PUCCINELLIA HOWELLII (POACEAE) IS GENETICALLY DISTINCT

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ABSTRACT

Population Aggregation Analysis of isozyme variation patterns among North American populations of *Puccinellia* (Poaceae), independent of prior notions of species identity, results in the delimitation of several distinct species, each comprising one or more populations. One of the solitary populations resolved as a distinct isozyme species is the Shasta Co., California, population previously recognized as the sole known population of *Puccinellia howellii* J. Davis. Isozyme variation patterns thus provide independent confirming evidence that this species is distinct.

Every valid description of a new plant species includes a diagnosis, i.e., a summary of the characters by which that species can be distinguished from others. Although the practice of species diagnosis predates the acceptance of organic evolution, it is as relevant today as ever. In the analysis of populations and multi-population systems of sexually reproducing organisms, genetically fixed differences—diagnostic character combinations—constitute direct evidence that one such system is evolutionarily independent of another (Eldredge and Cracraft 1980; Nelson and Platnick 1981; Cracraft 1983, 1989; Nixon and Wheeler 1990; Davis and Nixon 1992). These authors have used the term "phylogenetic species" to refer to population systems that are demarcated by fixed differences, and therefore are the least inclusive entities among which there is character evidence for hierarchic (i.e., non-reticulating) descent, and among which phylogenetic analysis is thus appropriate. Phylogenetic species are not discovered through phylogenetic analysis; like the characters in an analysis, phylogenetic species are basic elements of a phylogenetic analysis, and must be delimited by some other procedure prior to the analysis (see below, and see Davis and Nixon 1992). Thus, the venerable concept of species diagnosis is as significant in terms of evolutionary and phylogenetic theory as it is in the traditional practice of taxonomic species delimitation.

Puccinellia Parl. is one of the more controversial genera in the grass family in terms of species delimitation. Floristic treatments often identify complexes of questionably distinct species (e.g., Hughes and Halliday 1980), and different authors often propose quite dissimilar treatments of the species in the same region, even when they recognize similar numbers of species (e.g., Swallen 1944; Sørensen

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1968; Welsh 1974). It has been suggested (e.g., Hitchcock 1969; Welsh 1974) that difficulties encountered in delimiting species of *Puccinellia* are attributable to cleistogamy, which, by enhancing inbreeding, leads to genetic differentiation among populations; and to polyploidy, which helps to stabilize distinctive character combinations.

It is one thing, however, to propose which evolutionary factors have promoted a given pattern of populational differentiation, and another to determine whether a particular group of populations should be recognized as constituting one or more than one species. Objective analysis of existing patterns, as evident in different character sets, can reveal degrees of differentiation, and points of congruence (Mickevich 1978). Isozymes represent a large set of relatively independent and objectively scorable characters (Gottlieb 1977). They have often provided evidence of differentiation between morphologically cryptic and near-cryptic populations and population systems, whether or not these groups were regarded as species (e.g., Wolff and Jefferies 1987; Bruederle et al. 1989; Paris et al. 1989; Gottlieb and Edwards 1992). One objective method for delimiting species according to the criteria of the phylogenetic species concept is Population Aggregation Analysis (PAA; Davis and Manos 1991; Davis and Nixon 1992). PAA aggregates populations into diagnosable units (i.e., phylogenetic species) using any sort of character that can be scored as absent, present and fixed, or present and not fixed within each population. The analysis of any particular character set is conducted independently of previously recognized species boundaries, and PAA therefore generates independent assessments of species boundaries, which in turn allows for tests of congruence between different data sets.

I recently described *Puccinellia howellii* J. Davis in these pages (Davis 1990). This species, known from a series of mineral seeps in Shasta County, and first noted as unique by J. T. Howell, was initially diagnosed, like most vascular plant species, in terms of morphological characters. Here I present evidence of congruence between isozyme characters and the morphological characters that originally justified the recognition of *P. howellii.*

Materials and Methods

The present study is part of an ongoing analysis of isozyme variation in *Puccinellia* (Davis and Manos 1991; Davis and Goldman 1993). The sole known population of *Puccinellia howellii* was sampled by collection of seed (i.e., caryopses) from different individuals into separate envelopes. Seeds from these envelopes were sown in separate clay pots and later thinned to leave one plant per pot; the 45 individuals surveyed for isozyme variation therefore represent the offspring of 45 different individuals from the collection site. Seed

TABLE 1. ISOZYME ALLELE PROFILES OF FOUR ISOZYME SPECIES OF *PUCCINELLIA* IN CALIFORNIA (SEE TEXT). Eighty-three alleles are numbered separately within each diploid locus; 0 = absent; 1 = present, fixed; * = present, not fixed. Fixed differences between species are underlined.

	Aat			*Adh*	*Dia*	*Idh*	*Mdh*	
	1	*2*	*3*	*1*	*1*	*1*	*1*	*2*
	1234	1234	123456	123456	1234	1234	1234	12
P. howellii	0*10	0100	000100	**0100	0110	0100	0101	01
P. lemmonii	0**0	***0	0*****	00***0	****	0010	**0*	**
P. nuttalliana—1	0110	01*0	0*010*	0*0*00	0*10	0100	010*	01
P. nuttalliana—2	0110	*100	010101	*00100	0110	0110	*101	01

germination, enzyme electrophoresis, and staining of 17 diploid loci (i.e., distinct loci in related diploid species) were conducted as described by Davis and Manos (1991).

Population variation is summarized as a profile in which each allele observed in any population of *Puccinellia* is represented for each population as absent, present and fixed, or present and not fixed (Table 1). The profile of the Shasta Co. population has been analyzed in association with those of more than 100 other populations of North American *Puccinellia* in a single Population Aggregation Analysis (Davis and Manos 1991; Davis and Goldman 1993; Davis unpublished data). As noted above, PAA determines the number and membership of distinct species recognizable on the basis of population variation profiles, and thus provides an independent test of congruence with species boundaries that have been recognized on the basis of other attributes (Davis and Manos 1991; Davis and Nixon 1992). In brief, all populations, regardless of presumed identity, were compared with all others. Any two populations that were not distinct in at least one character (i.e., fixation of an allele in one and absence of the allele in the other) were aggregated (i.e., grouped), and a summary profile of the group replaced those of the constituent populations. Cycles of aggregation were continued until all groups and all ungrouped populations were distinct, and these were recognized as putative isozyme species.

RESULTS

Staining with eleven substrate systems led to the resolution of seventeen presumptive loci among the diploid species of *Puccinellia,* each apparently present in multiple copies among the remaining species, most of which are known to be polyploids (Davis and Manos 1991; Davis and Goldman 1993). Members of the latter group,

TABLE 1. CONTINUED.

Mdh	*6-Pgd*		*Pgi*	*Pgm*	*Skdh*	*Sod*	*Tpi*	
3	*1*	*2*	*2*	*1*	*2*	*1*	*1*	*2*
			11					
1234567	1234	12345	12345678901	123456	123456	123	1234	123
0010000	1100	11000	0001010001*	*10*00	**1000	010	0010	010
0̄0*00*0	**00	*̄***0	00**0***0*0	0*0***	00̄0***	0**	0̄**	***
0̄**00*0	1100	01*00	**010*00000	010*00	00*̄1*0	010	0*̄10	0**
1̄010000	1100	0̄1000	000*0*00*0̄0	010*00	0001̄10	010	011̄0	01*

including *P. howellii,* exhibit the characteristic "fixed heterozygosity" pattern typical of polyploid species (Roose and Gottlieb 1976; Gottlieb 1977). Although a chromosome count has not been obtained for *P. howellii,* the sampled population exhibits at least one fixed allele at each diploid locus, two fixed alleles at each of four loci, and three fixed alleles at one locus, *Pgi2* (Table 1).

With more than 100 populations of North American *Puccinellia* now incorporated into the ongoing population aggregation analysis, the solitary population of *P. howellii* continues to be resolved as distinct. Among other species represented in the analysis are *P. lemmonii* (Vasey) Scribner and *P. parishii* A. Hitchc., the only two diploid species in temperate North America (Davis and Goldman 1993); the predominantly inland *P. nuttalliana* (Schultes) A. Hitchc. complex, within which six isozyme species are resolved (Davis and Manos 1991); the predominantly coastal *P. pumila* (Vasey) A. Hitchc. complex (Davis unpublished data), within which five isozyme species are resolved; and *P. distans* (L.) Parl., *P. fasciculata* (Torrey) E. P. Bicknell, and *P. simplex* Scribn. (Davis unpublished data).

For purposes of comparison, the isozyme profile of *P. howellii* is presented with those of three other western North American species with isozyme profiles similar to that of *P. howellii* (Table 1), and likely to be among its closest relatives. Each of these profiles of a related species represents several populations that have become aggregated during the course of the analysis. *Puccinellia howellii* is distinct from *P. lemmonii* and from isozyme species 1 and 2 of *P. nuttalliana* in 3, 3, and 10 fixed differences, respectively. At least three fixed differences also have been identified between *P. howellii* and every other species of *Puccinellia* that has been delimited, including *P. pumila,* which is similar to it in morphology (Davis 1990; Davis and Manos 1991; Davis and Goldman 1993; Davis unpublished data). *Puccinellia lemmonii,* a diploid species, exhibits a great-

er number of alleles than any other species sampled in this study. To the extent that it has been sampled, *P. lemmonii* appears to be fixed for just one allele (*Idh1,* allele 3), which is absent from *P. howellii*; conversely, *P. howellii* is fixed for two alleles that have not been observed in *P. lemmonii* (*Idh1,* allele 2; and *Skdh2,* allele 3), each of which is present in at least one of the isozyme species of *P. nuttalliana* (Table 1). *Puccinellia howellii* also is fixed for two alleles that do not occur in either of the *P. nuttalliana* isozyme species (*6Pgd2,* allele 1; and *Pgi2,* allele 10), and both of these alleles occur in *P. lemmonii.* Although every allele that is fixed in *P. howellii* and absent from one of the other two species in Table 1 is also present in some other species, *P. howellii* also carries two nonfixed alleles (*Pgi2,* allele 11; and *Skdh2,* allele 1) that have not been detected in any other population of *Puccinellia.*

Discussion

There is considerable interest in the reconstruction of descent relationships among species, as there is in the process of speciation. In recent years phylogenetic analysis has developed into a formal, repeatable methodology involving character state definition, parsimony analysis, and related procedures. There has been less progress, however, towards universally accepted procedures for the delimitation of species. It should be obvious, however, that the results of phylogenetic analyses, as well as those of studies of speciation, introgression, and other processes, are influenced by the initial apportionment of individuals and populations among species. In short, species delimitation should be conducted by explicit procedures; the development of Population Aggregation Analysis is an attempt to achieve this objective. As with other analytical procedures employed in systematics, including phylogenetic analysis, the results obtained reflect the organisms and characters that are sampled, and undersampling introduces predictable biases (Davis and Nixon 1992). Thus, all results are provisional, and subject to the collection of additional data.

However many species an investigator chooses to recognize within *Puccinellia,* the observed situation remains that of a polyploid complex with relatively few diploid species, with pronounced genetic structuring evident within many local populations (Davis and Manos 1991; Davis unpublished data), and with some distinct assortments of isozyme alleles occurring repeatedly across several populations, while other combinations occur in one or only a few populations. The latter situation is exemplified by the Shasta Co. population that has been recognized as *P. howellii.* Thus, the available evidence is consistent with the delimitation of this population as a phylogenetic species, *Puccinellia howellii.* It is resolved as a single isozyme species,

distinct from all other populations sampled, precisely in congruence with its previous delimitation on the basis of morphology.

Every allele that appears to be fixed in *Puccinellia howellii* has been observed elsewhere in the genus, but because this species also carries two unique alleles (neither of them fixed), its isozyme profile cannot be assembled by the summing of those of any known combination of other species. *Puccinellia howellii* may have originated by divergence from a single ancestral species (cf. Gottlieb 1973), or via interspecific hybridization, and it is difficult to rule out either possibility. Under either speciation model the two unique alleles in *P. howellii* might have arisen either before or after the origin of this species. All other alleles known to occur in *P. howellii* also have been observed elsewhere in the genus, but not as a group within any single species. If it is assumed that all occurrences of comigrating enzyme electromorphs (i.e., those that migrate identical distances under the electrophoretic conditions employed) do represent identical alleles, and that all cases of shared alleles between species have been discovered, the hypothesis of speciation without hybridization still cannot be dismissed unless it is further assumed that related species have not lost alleles they once shared with *P. howellii.*

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ANNOUNCEMENT

On 3 August 1993, Dr. Alwyn Gentry, a member of the Missouri Botanical Garden research staff for 21 years, was killed in an airplane crash in western Ecuador. He was 48 years old and leaves behind his wife, Rosa, and three children. He has long been recognized for his knowledge and collections of New World Tropical floras.